

WHAT IS CLAIMED:

- Sub 13*
1. A method of characterizing single cells comprising the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in a single cell using fluorescent microscopy.
- mb*
Cl 7
2. The method of claim 1, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each specific marker.
3. The method of claim 1, wherein the cell is isolated from a body fluid.
4. The method of claim 1, wherein cells are isolated from a body fluid using gradient density centrifugation.
- Arb*
B1
5. The method of claim 3, wherein said body fluid is selected from the group consisting of blood, bone marrow, saliva, cerebrospinal fluid, urine, a body cavity fluid, and semen.
6. The method of claim 1, wherein said cell is a white blood cell.
7. The method of claim 1, wherein said cell is a cancer cell.
8. The method of claim 7, wherein said is a circulating cancer cell.
9. The method of claim 1, wherein the surface for cell adherence is a microscope slide. *LOA*
- Arb*
B2
10. The method of claim 1, wherein the fixative is selected from a group consisting of paraformaldehyde, formaldehyde, alcohol, or acetone. *LOA*
11. The method of claim 1, wherein said probe is covalently linked to a fluorescent compound that emits a wavelength of light to create a fluorescent probe that binds to a cellular marker.
12. The method of claim 11, wherein said fluorescent probe is selected from other probes with minimal overlapping emission spectra for concurrent use in characterizing said single cell.
- mb*
Cl 1
Arb
13. The method of claim 12, wherein said fluorescent probes are selected from a group consisting of a mixture of fluorescent probes that emit light of wavelengths between 400 nanometers and 850 nanometers, wherein said emission spectra can be distinguished from each other with the use of a microscope equipped with spectral filters that allow for

elimination of most overlapping wavelengths of fluorescent light being emitted by each selected probe.

14. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 430 nanometers to 510 nanometers.

15. The method of claim 14, wherein said fluorescent probe emits light with a peak wavelength of about 470 nanometers.

16. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 482 nanometers to 562 nanometers.

17. The method of claim 16, wherein said fluorescent probe emits light with a peak wavelength of about 522 nanometers.

18. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 552 nanometers to 582 nanometers.

19. The method of claim 18, wherein said fluorescent probe emits light with a peak wavelength of about 567 nanometers.

20. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 577 nanometers to 657 nanometers.

21. The method of claim 20, wherein said fluorescent probe emits light with a peak wavelength of about 617 nanometers.

22. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 637 nanometers to 697 nanometers.

23. The method of claim 22, wherein said fluorescent probe emits light with a peak wavelength of about 667 nanometers.

24. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 730 nanometers to 814 nanometers.

25. The method of claim 24, wherein said fluorescent probe emits light with a peak wavelength of about 772 nanometers.

26. The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 745 nanometers to 845 nanometers.

27. The method of claim 26, wherein said fluorescent probe emits light with a peak wavelength of about 795 nanometers.

28. The method of claim 13, wherein fluorescent compounds are selected from a group consisting of fluorescein isothiocyanate; CY3; CY3.5; CY5; CY5.5; AMCA; Tetramethylrhodamine Isothiocyanate; TEXAS RED™; R-Phycoerythrin; and Spectral Red.

29. The method of claim 1, wherein the probes comprise 4 fluorescent probes.

30. The method of claim 1, wherein the probes comprise 5 fluorescent probes.
31. The method of claim 1, wherein the probes comprise 6 fluorescent probes.
32. The method of claim 1, wherein the probes comprise 7 fluorescent probes.
33. The method of claim 1, wherein the probes comprise multiple fluorescent probes that emit light of different wavelengths with minimal interference between the wavelengths of emitted light when using appropriate filter set combinations that allow one marker to be distinguished from another when tested concurrently.
34. The method of claim 1, wherein said probe comprises a biological probe.
35. The method of claim 34, wherein said biological probes comprises a protein or a peptide.
36. The method of claim 35, wherein said protein is an antibody.
37. The method of claim 1, wherein said probe comprises a molecular probe.
38. The method of claim 37, wherein said molecular probe comprises DNA or a DNA sequence thereof.
39. The method of claim 38, wherein said molecular probe comprises RNA or an RNA sequence thereof.
40. The method of claim 1, wherein said probes comprise biological probes, molecular probes, or a combination of biological and molecular probes.
41. The method of claim 40, wherein the biological probes are selected from a group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes, viral, bacterial and hormonal probes.
42. The method of claim 40, wherein the molecular probes are selected from a group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes, viral, bacterial and hormonal probes.
43. The method of claim 40, wherein said probes comprise an epithelial cell-specific probes.
44. The method of claim 40, wherein the probes comprise a tissue-specific probes.
45. The method of claim 1, wherein said cell is obtained from a mammal.
46. The method of claim 45, wherein said mammal is a human.
47. The method of claim 40, wherein said biological and molecular probes are used to detect a hormone receptor or a hormone receptor gene for the enumeration of copy number.
48. The method of claim 47, wherein said hormone is an androgen.

49. The method of claim 47, wherein said hormone is an estrogen.

50. The method of claim 47, wherein said hormone is a progesterone.

51. The method of claim 1, wherein said cellular marker is an antigen.

52. The method of claim 51, wherein said cellular marker is a receptor.

53. A method of characterizing a single cell preparation comprising adhering a cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating such a cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescent microscopy for identification of positive cells for each selected cellular marker, wherein said cancer cell preparation is isolated from a body fluid sample using a negative selection process.

54. A method of establishing a characterization profile comprising a method of characterizing a single cell environment, wherein the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in a single cell using fluorescent microscopy.

55. The method of claim 53, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each individual marker.

56. The method of claim 4, wherein cells are further isolated by a negative selection process.

57. The claim of 56, wherein said target cell is a cancer cell.

58. The method of claim 4, wherein cells are further isolated by a positive selection process, wherein a specific cell type is selected from a heterogeneous mixture of cells by an antibody that selectively binds to cell.